



ENZYMIC HISTOCHEMISTRY OF GRANULAR COMPONENTS IN DIGESTIVE GLAND CELLS OF THE ROMAN SNAIL, *HELIX POMATIA*¹

ROBERT M. ROSENBAUM AND BRUCE DITZION

Department of Pathology, Albert Einstein College of Medicine, New York 61, New York

The digestive gland of *Helix pomatia* has been studied histologically especially during feeding and digestion (Krijgsman, 1925, 1929; Thiele, 1953; Guardabassi and Ferreri, 1953 among others). In addition, biochemical studies employing homogenates of gland tissue or aliquots of crop or digestive fluid have established that both intra- and extracellular hydrolytic enzymes are associated with the gland tissue (Holden and Tracey, 1950). Such methods, while providing quantitative data, do not permit direct study of the intracellular enzymic activity within the gland. *Helix* appears able to produce enormous amounts of several hydrolytic enzymes rapidly (Holden and Tracey, 1950; Billett, 1954; Dodgson and Powell, 1959) and it is therefore not unreasonable to assume that a highly developed synthetic machinery for secretion of extracellular hydrolases may exist within the cells concerned.

The activity of several hydrolytic enzymes, including acid phosphatase, β -glucuronidase, and several "cathepsins," has been shown by biochemical methods to vary according to the feeding cycle (Holden and Tracey, 1950; Jarrige and Henry, 1952). Several histochemical studies have shown β -glucuronidase activity (Billett and McGee-Russell, 1955) and acid and alkaline phosphatase activity (Guardabassi and Ferreri, 1953; Nakazima, 1956) within digestive gland tubules. To date, no reports have dealt with visualization of enzymic activity within specific digestive gland cells.

In this investigation we were concerned not only with achieving intracellular localization of specific hydrolases, but also with a comparison of intracellular enzymic activity during periods of starvation and active feeding. We were especially interested in the identification of enzymic activity with specific intracellular granules. The position of these granules has been shown to vary during feeding and digestion (Krijgsman, 1929; Rosen, 1941). With cytochemical methods, we were hopeful that it would be possible to extend these earlier cytological observations and establish a more precise role for these granules consistent with some recent concepts of intracellular digestion and related hydrolytic enzymic activity.

MATERIALS AND METHODS

Our initial stock culture consisted of mature, estivating specimens of *Helix pomatia* from Morocco.² The snails were activated by exposure to a warm, humid

¹ This study was supported by grants from the United States Public Health Service (RG-5483 and A-3605) and a contract with the Office of Naval Research.

² Courtesy of Miscuraca Importing Corp., New York, N. Y.

environment. Animals were deemed "fed" after they had been observed to feed continuously on fresh lettuce leaves for several hours following starvation for 5–7 days. Animals were considered "starved" when they had been isolated in individual fingerbowls for at least 7 days without food, or when they were killed while in estivation. For some experiments, animals were fed for at least 24 hours on fresh lettuce leaves soaked in a solution (25 mg. per 1 ml. water) of horse-radish peroxidase.

The digestive gland was located by removing the apex of the shell and uncoiling the animal, the gland being identified as a green-brown mass near the upper end of the coil. For histochemical purposes, tissue was cut into small pieces (1–2 mm.) immediately following removal and fixed in cold (4° C.) calcium-formalin (Baker's) or cold chloral hydrate formalin (Fishman and Baker, 1956) for 18–24 hours. Other pieces of gland tissue were fixed in aqueous Bouin's fluid, alcohol-formol-acetic acid fixative or Carnoy's fluid.

Prior to being sectioned on a freezing microtome, digestive gland tissue must be embedded in gelatin, owing to its friable nature. Fixed tissue was washed briefly in cold water and placed for no longer than one hour in 15% gelatin at 37° C. and then hardened at 0° C. for 20 minutes, and by additional treatment in cold 10% neutralized formalin for one hour. Immediately prior to sectioning, the block was briefly rinsed in cold water.

Enzymes: *Acid phosphatase* activity was visualized by the lead-salt method of Gomori (1952), with β -glycerophosphate as substrate, and by the azo dye method of Burstone (1958) with naphthol AS-MX phosphate as substrate and Red Violet LB as coupling reagent following cold acetone treatment (20 minutes) to remove lipid. With both methods, sections were incubated for 20–60 minutes at 37° C. (Rosenbaum and Rolon, 1962). For visualization of *β -glucuronidase* activity, we employed cold chloral-hydrate-formalin-fixed tissue with 8-hydroxyquinoline glucuronide as substrate (Fishman and Baker, 1956). Sections were incubated at 37° C. for 30 minutes to 6 hours. For *non-specific esterase* activity, the pararosanilin method of Lehrer and Ornstein (1959) was used following exposure of fixed sections to cold acetone. Incubation proceeded at 0° C. for 30 minutes to two hours with α -naphthyl acetate as substrate. A parallel series of tissues was exposed to the organophosphorous compound E-600 (diethyl-*p*-nitrophenyl phosphate, 10^{-5} M in Tris-maleate buffer, pH 7.2) for one hour at 37° C. prior to incubation. This procedure has been considered capable of demonstrating type-C esterases (Hess and Pearse, 1958). For localization of *aminopeptidase* activity, we employed the method of Burstone and Folk (1956), using the substrate L-leucyl β -naphthylamide at pH 7.1 (0.2 M Tris buffer) following treatment of sections in cold acetone. Simultaneous coupling was obtained with the diazonium salt, Garnet GBC.

Other histochemical methods: Detection of phospholipid was performed with the acid hematein method of Baker (1946). Some paraffin-embedded tissues fixed in Bouin's and Carnoy's fixatives were stained by the periodic acid Schiff method following digestion with salivary amylase. Finally, visualization of exogenous peroxidase, employed as a "marker" in some experiments, was accomplished by use of a hydrogen peroxide substrate and benzidine, essentially as described by Straus (1959).

RESULTS

Cytology of the digestive gland: Several authors have presented good descriptions of the kinds of cells in the digestive gland of *Helix* (Krijgsman, 1929; Rosen, 1941; Thiele, 1953). This work has resulted in a variety of terms frequently descriptive of the same cell type. It is desirable, therefore, to clarify our nomenclature for the cell types considered in the present study.

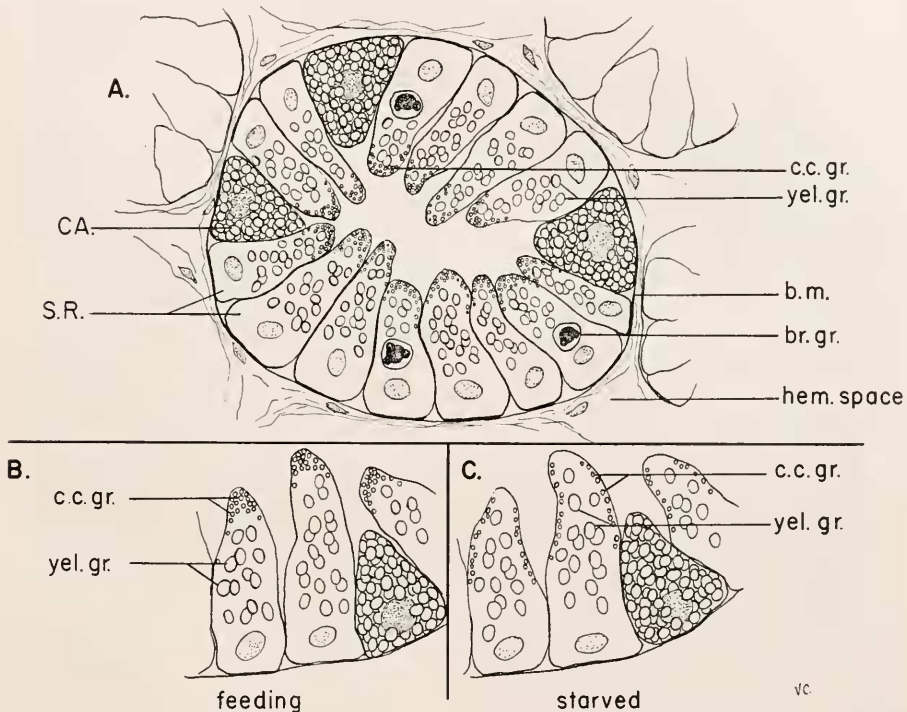


FIGURE 1. A. Schematic drawing of a digestive gland tubule from *Helix pomatia*, showing relationships of the different cytological components. B. Distribution of various intracellular granules during the feeding stages of *Helix*. Clear colorless granules are almost exclusively localized at the luminal border of the secretory-resorption cells. C. In secretory-resorption cells from starved animals, the clear, colorless granules are not concentrated at the luminal border. CA, calcareous cell; SR, secretory-resorption cell; c.c. gr., clear, colorless granules; yel. gr., yellow granules; b.m., basement membrane; hem. space, hemocoelic space.

Our observations concern two kinds of cells (Fig. 1)—the so-called *calcium cell* and the *digestive* or *SR cell* (secretory-resorption cell—Rosen, 1941). The *calcium cell* is characterized by its triangular shape with the broad base (approximately $50\ \mu$) touching the basement membrane. The cell points toward, but does not extend into, the tubular lumen. The cytoplasm contains numbers of large, nearly colorless spherules, the so-called *calcium spherules*. *Secretory-resorption (SR) cells* are greater in number than, and adjacent to, the calcium cells. The SR cells are long ($100\ \mu$), the body of the cell extending from the basement mem-

brane into the lumen of the glandular tubule. The nucleus is generally smaller than that of the calcium cell. The SR cell possesses granular inclusions, the number and distribution of which vary considerably with the feeding stage of the animal (Krijgsman, 1929). These granules are generally described according to three types: *large brown granules* not present in all cells, but, when present, con-

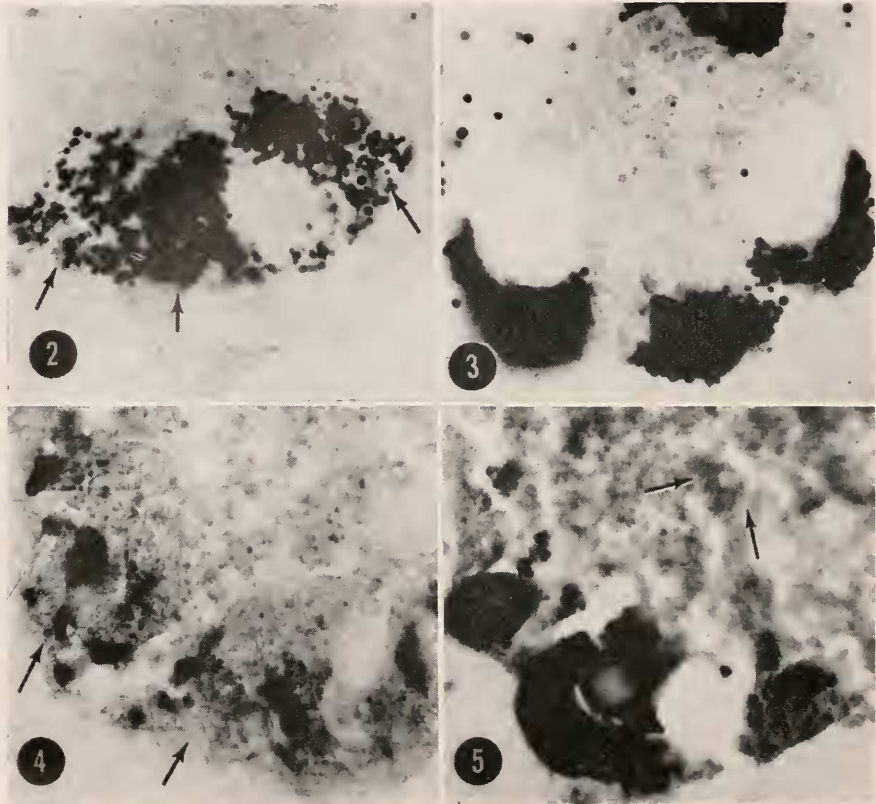


FIGURE 2. Three calcium cells (arrows) from the digestive gland of a starved snail, stained with the method of Gomori, omitting glycerophosphate from the incubation medium. Calcium spherules are stained by lead sulfide. All other regions of the digestive gland are negative. 360 \times .

FIGURE 3. Four calcium cells from the digestive gland of a recently fed snail, stained as in Figure 2 but with glycerophosphate present as substrate. Calcium spherules, stained by the false positive reaction, are obscured by an intense cytoplasmic staining due to enzymic activity. A few spherules have been scattered extracellularly in processing. 360 \times .

FIGURE 4. Tubule from the digestive gland of a starved animal, stained for acid phosphatase activity by the azo dye method of Burstone. Enzymic activity is present in calcium cells (arrows), and weaker activity is present in the cytoplasm of SR cells. Spherules do not stain. Green filter. 360 \times .

FIGURE 5. Tubule from the digestive gland of a recently fed animal, stained as in Figure 4. Intense enzyme activity is seen in the cytoplasm of three calcium cells. The spherules do not stain. The cytoplasm of the SR cells is heavily stained, especially near the lumen (arrows). Green filter, 360 \times .

tained within vacuoles located at the base of the cell in the vicinity of the nucleus; *small yellow granules* generally distributed throughout the cytoplasm; *small clear granules* apparently free in the cytoplasm near the periphery of the cell. The distribution of all granule types is summarized in Figure 1A.

Enzyme distribution within the gland cells of starved and feeding animals

Acid phosphatase: With the Gomori metal-salt method, we could not distinguish acid phosphatase activity in the cytoplasm of calcareous cells since calcium spherules from both starved and feeding animals blackened intensely with the sulfide. These calcium granules were large, round bodies, which frequently became dispersed

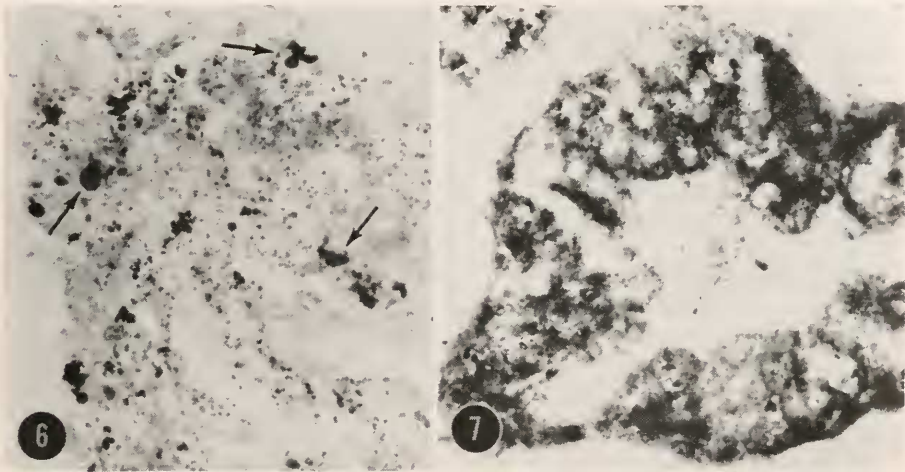


FIGURE 6. Digestive gland tubules from a starved animal, stained for β -glucuronidase activity by the method of Fishman and Baker, following fixation in cold chloral-hydrate-formalin. Enzymic activity is confined to the yellow granules. Brown granules (arrows) did not stain for enzymic activity but are emphasized by the yellow filter employed. Calcium cells are negative. 200 \times .

FIGURE 7. Digestive gland tubule from a recently fed animal, stained as in Figure 6. There is increased cytoplasmic activity, especially at the periphery of SR cells. The yellow granules are also stained. Calcium cells are negative. Yellow filter. 200 \times .

outside the cell due to compression of the sections during processing (Figs. 2 and 3). Application of heat to the sections (90° C. for at least 10 minutes), or omission of glycerophosphate substrate from the incubation medium, demonstrated that this localization of final reaction product was a false positive reaction not due to enzymic activity. With use of an azo dye method for acid phosphatase activity, the spherules showed no enzymic activity (Figs. 4 and 5). With staining by the azo dye method, however, it was clear that the cytoplasm of calcareous cells from recently fed animals showed increased acid phosphatase activity over cells from starved animals (compare Figs. 4 and 5).

The SR cells from starved animals showed little or no enzymic activity by either the azo dye or lead-salt methods. In fed animals, some enzymic activity could be detected near the luminal border in the region where the small, colorless

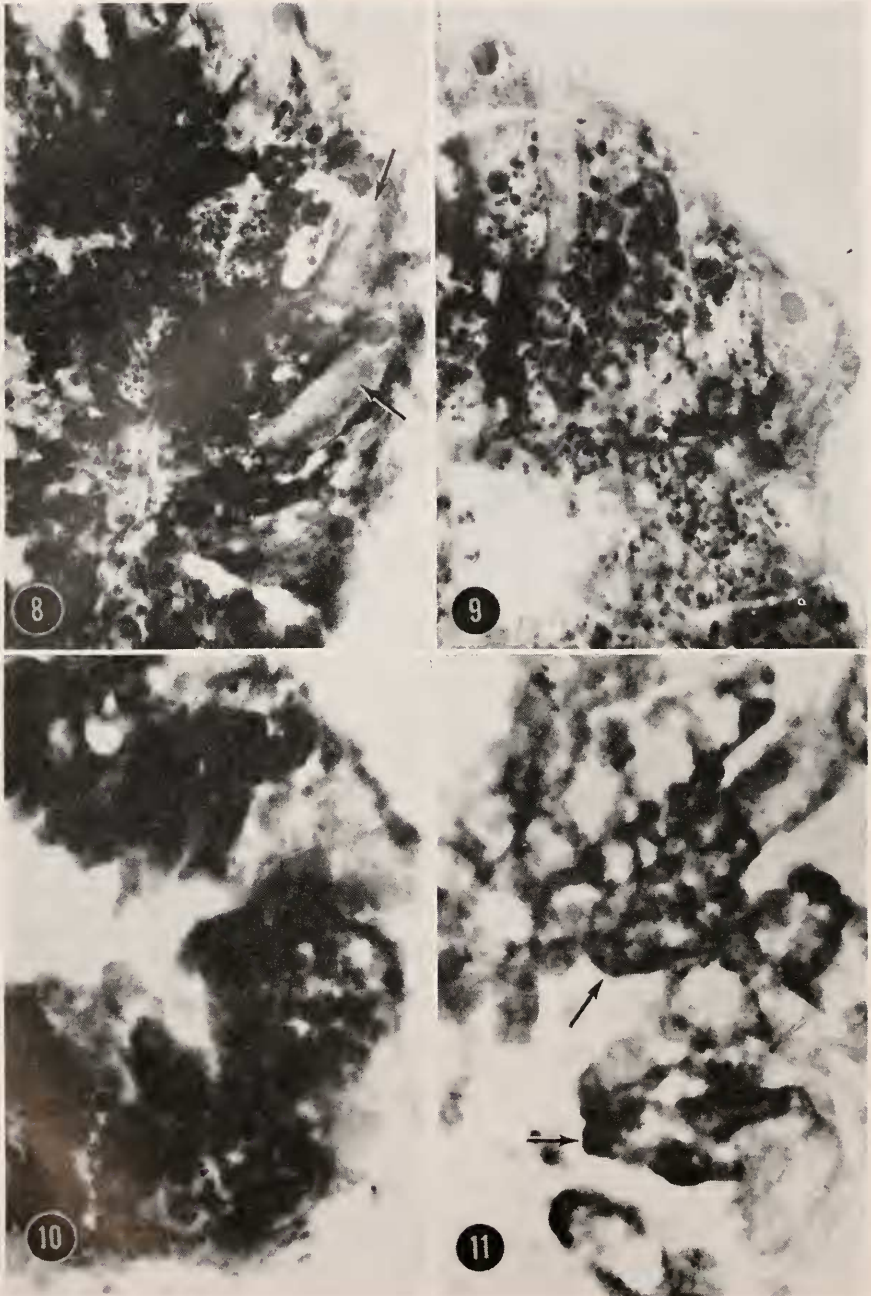


FIGURE 8. Digestive gland tubule from a starved animal, stained for non-specific esterase activity by the method of Lehrer and OrNSTEIN. There is intense cytoplasmic staining, and staining of granules is evident in SR cells. Calcium cells (arrows) are negative. Green filter, 380 \times .

granules accumulated. Neither the small yellow nor large brown granules possessed demonstrable acid phosphatase activity by the methods we employed.

When sections were exposed to the non-ionic surface-activating agent, Triton X-100 (0.25% at 4° C. for one hour), a distinct decrease of enzymic activity within calcium cells could be detected with the azo dye method, especially in tubules from starved animals. With identical treatment applied to tissue from feeding animals, enzymic activity in calcium cells and at the lumen of SR cells was also diminished.

β -Glucuronidase (β -glucosiduronidase): Calcium cells showed no activity for this enzyme. In SR cells from starved animals, intracellular enzymic activity could be detected in locations approximating that of the yellow granules (Fig. 6). The large brown granules showed no enzymic activity. Diffuse staining of the peripheral cytoplasm occurred in locations consistent with those of the small colorless granules. In secretory-resorption cells from fed animals, staining for β -glucuronidase activity was intense and generally distributed throughout the cytoplasm. Activity for this enzyme also appeared in the yellow granules (Fig. 7).

Esterases: Calcium cells from both starved and feeding animals showed no esterase activity (Figs. 8 and 9). Secretory-resorption cells stained intensely for non-specific esterase activity. Activity appeared throughout the cytoplasm (Fig. 8), but with fed animals the reaction was more intense and diffuse (Fig. 10) than with starved animals. In all cases, staining for non-specific esterase activity could not be identified with specific granules.

In both starved and feeding animals, treatment with the organo-phosphorous inhibitor E-600 resulted in a considerable loss of cytoplasmic activity. In preparations from starved animals, E-600-resistant esterase activity was limited to what appeared to be the yellow granules of the SR cells (Fig. 9). Treatment of sections from fed animals in E-600 resulted in a diffuse localization of enzymic activity within peripheral regions of SR cells (Fig. 11). This region generally contained the small, colorless granules.

Aminopeptidase: Calcium cells from both starved and feeding animals showed no enzymic activity. Some slight activity was detected in secretory-resorption cells from starved and feeding animals.

Other histochemical methods

Phospholipid: With Baker's acid hematein method for phospholipid, positive staining occurred in the peripheral region of SR cells from starved and feeding animals (Figs. 12 and 13). Localization of phospholipid in SR cells in the glands of starved animals was more diffuse (Fig. 12), while in fed animals it was limited

FIGURE 9. Digestive gland tubule from a starved animal, stained as in Figure 8, following treatment of the section in the organo-phosphorous inhibitor E-600. Non-specific enzyme activity present in the cytoplasm of cells in Figure 8 has been removed but enzymic activity remains in yellow granules scattered throughout the SR cells. Calcium cells are negative. Green filter. 380 \times .

FIGURE 10. Digestive gland tubule from a feeding animal, stained for non-specific esterase activity as in Figure 8. There is intense enzyme activity in SR cells. Green filter. 380 \times .

FIGURE 11. Digestive gland tubule from a recently fed animal. The section was treated with E-600 and stained for esterase activity as in Figure 9. While general cytoplasmic enzyme activity is diminished, there is positive staining for E-600-resistant enzyme activity near the lumen of the tubule (arrows). The calcium cells are negative. Green filter. 380 \times .

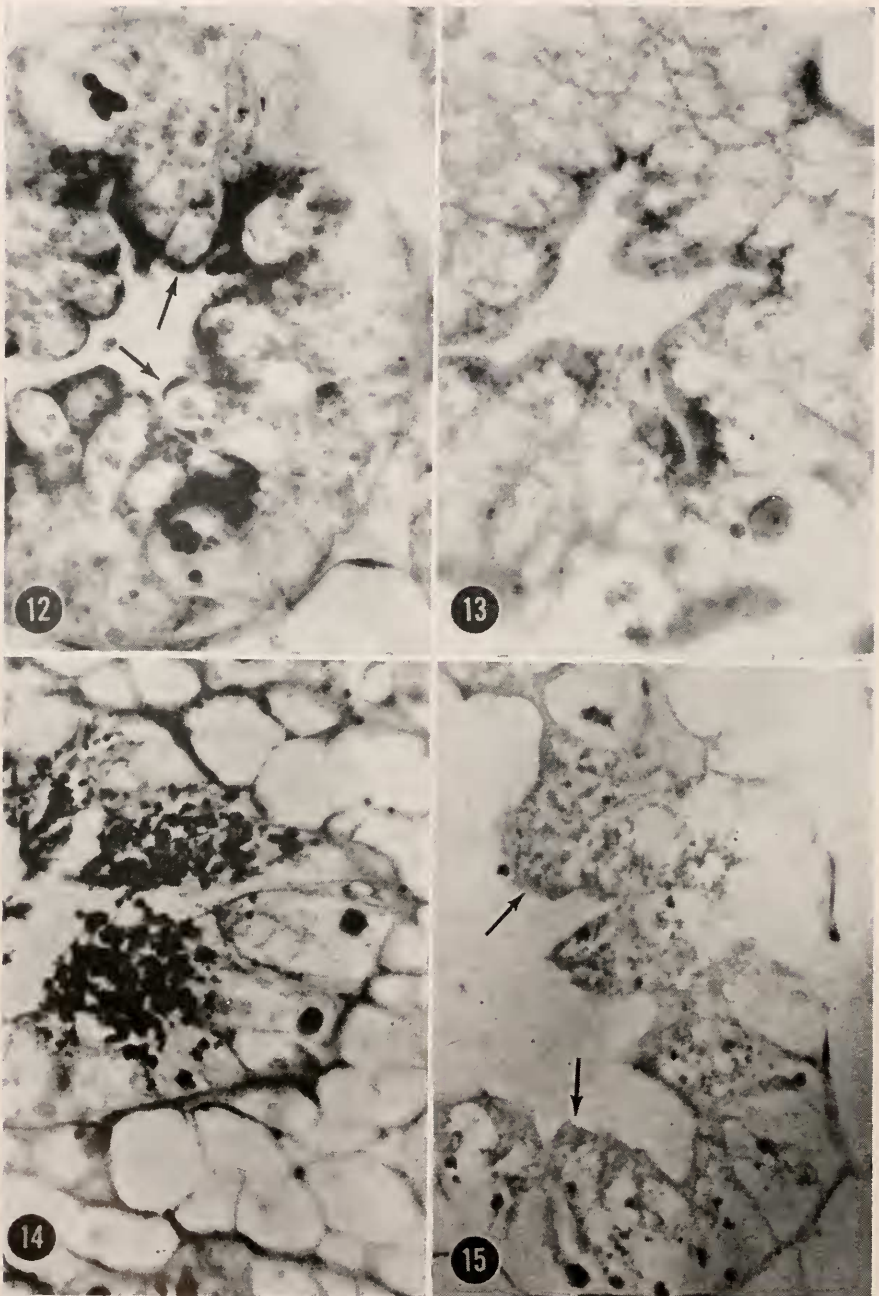


FIGURE 12. Tubule from the digestive gland of a starved animal, stained for phospholipid by the method of Baker. There is peripheral staining of SR cells, both at the luminal border (arrows) and the lateral cell margins. Brown granules, although detectable, are not stained with this method. 360 \times .

to the luminal border (Fig. 13). The location of phospholipid staining corresponded to areas containing the small, colorless granules. A weak reaction for phospholipid was present throughout the cytoplasm of SR cells in both starved and feeding animals. There was no staining of the calcium cells.

PAS reaction: The periodic acid Schiff reaction was employed following incubation with amylase at pH 7.4 for digestion of glycogen. In secretory-resorption cells from starved animals, the yellow granules were intensely Schiff-positive (Fig. 14). In cells from fed animals, fewer yellow granules were present and these showed a diminished Schiff reaction (Fig. 15). In glands from fed animals fixed

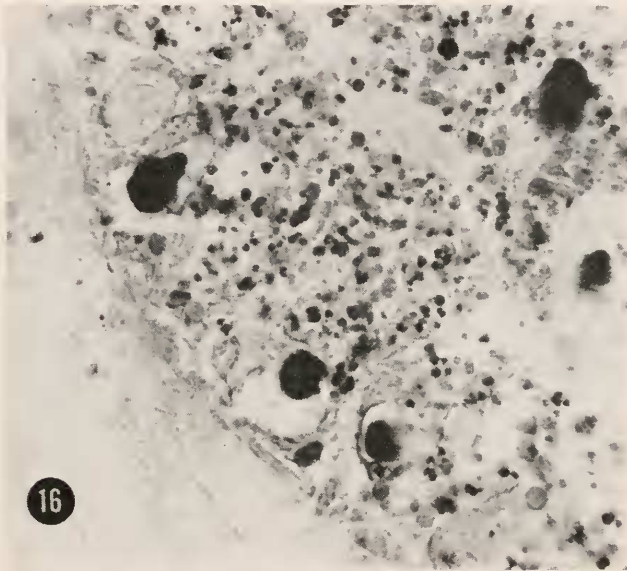


FIGURE 16. Digestive gland tubule from a snail fed on lettuce soaked in horse-radish peroxidase, visualized by means of a benzidine reaction. Numerous "vacuoles" with enzyme activity appear throughout SR cells. Brown granules are also stained by the benzidine reaction but do not possess enzyme activity. Yellow filter. 360 \times .

in aqueous Bouin's fluid, a weak Schiff-positive reaction appeared at the luminal border of the secretory-resorption cells (Fig. 15). This region corresponded to where localized phospholipid was detected and where, in fed animals, the small colorless granules were always observed.

Ingestion of peroxidase: Within 15 minutes following initial attachment of a snail onto a leaf of treated lettuce, peroxidase could be detected in the cytoplasm

FIGURE 13. Tubule from the digestive gland of a fed animal stained for phospholipid as in Figure 12. The reaction is present chiefly at the luminal border of SR cells. 360 \times .

FIGURE 14. Tubule from the digestive gland of a starved snail, fixed in Bouin's fluid and stained with periodic acid Schiff method following digestion with saliva. The yellow granules within SR cells appear intensely Schiff-positive. Green filter. 360 \times .

FIGURE 15. Digestive gland tubule from a fed animal, stained as in Figure 14. A few yellow granules are present but weakly stained. There is staining of the luminal border of SR cells, corresponding to the location of clear, colorless granules (arrows). Green filter, 360 \times .

of the secretory-resorption cells (Fig. 16). Localization of ingested enzyme appeared to be within "vacuoles" scattered throughout the cytoplasm, especially near the luminal border of the SR cells. The location of the larger peroxidase-positive "vacuoles" corresponded to that of the yellow granules. With extended feedings (up to three hours) on treated lettuce, we could detect no increase in the amount of peroxidase ingested, nor did existing sites of peroxidase activity coalesce to form larger vacuoles.

Control experiments on cells from animals fed untreated lettuce revealed no peroxidase activity endogenous to the snail itself.

DISCUSSION

In this study, hydrolase activity was localized within secretory-resorption cells and calcium cells from the digestive gland of *Helix*. The location of some of this enzymic activity closely corresponded to the distribution of migrating intracellular granules described by Krijgsman (1925, 1929) and by Rosen (1941).

The activities of the hydrolases considered here have previously been visualized primarily in cells from vertebrate tissues, where their role in resorption phenomena or intracellular digestion has been described. Thus, acid phosphatase activity has been identified within phagocytic macrophages (Weiss and Fawcett, 1953), with iron resorption in liver (Novikoff and Essner, 1960) and protein resorption in kidney (Straus, 1961). The activity of two other hydrolases has been visualized in the peribiliary region of vertebrate liver cells (esterase—Holt, 1956; β -glucuronidase—Goldfarb and Barka, 1960), where their role in resorption was suggested. Studies with invertebrates have identified intracellular hydrolytic activity with feeding and digestion in planarians (Rosenbaum and Rolon, 1960), amebas (Novikoff, 1959; Birns, 1960) and ciliate protozoa (Seaman, 1961; Rosenbaum and Wittner, 1962).

Unfortunately, those few studies describing the activity of hydrolytic enzymes in molluscan digestive glands (see review of Arvy, 1962) employed methods no longer deemed completely reliable for purposes of intracellular localization. In the present investigation, we attempted to increase reliability of the methods by minimizing enzyme diffusion and inactivation through use of frozen sections of tissue fixed briefly in the cold. Such methods, especially in combination with newer naphthol substrates and rapid simultaneous coupling to a diazonium salt, offered additional protection against diffusion of enzyme, final colored reaction product, or both (Rosenbaum, 1962). Control of these factors served to support our observations that much, if not all, of the intracellular enzymic activity we studied was associated with granular or with vacuolar structures within the cells concerned.

Within a short time following ingestion of food, the secretory-resorption cells showed increased activity for several enzymes, especially β -glucuronidase and esterases. Much of this activity was associated with yellow granules, although non-specific esterase activity was also diffusely distributed in the cytoplasm of secretory-resorption cells. It is noteworthy that the yellow granules also served as sites of exogenous horse-radish peroxidase accumulation. We propose that this enzyme entered the cell by pinocytosis, perhaps via the clear, colorless granules, which may represent small pinocytic vacuoles. The observation that these small granules stained for phospholipid and were also Schiff-positive suggests a possible relation-

ship to the cell membrane. During feeding, the region of accumulation of these clear, colorless granules also possessed increased E-600-resistant esterase activity.

The present observations did not permit determination as to whether the yellow granules in the secretory-resorption cells could form vacuolar structures associated with the granules as resorption took place. The accumulation of pinocytic vacuoles associated with migrating granular structures has been described in specialized vertebrate cells grown in tissue culture (Rose, 1957a, 1957b). In *Paramecium*, Rosenbaum and Wittner (1962) have described migration of neutral red-staining granules from a region beneath the pellicle toward forming food vacuoles as these become larger and begin to show increased activity for acid phosphatase, non-specific and E-600-resistant esterases during feeding. In addition to this enzymic activity, the neutral red bodies of the paramecium stained for phospholipid, and their possible relationship to vertebrate hepatic lysosomes (deDuve, 1959) was therefore suggested. We have made no observations with respect to the ability of granular components within the SR cells of *Helix* to stain selectively with neutral red.

The present study points also to the possibility that intracellular digestion may take place within secretory-resorption cells of the digestive gland. Phagocytosis, which is closely related to pinocytosis, occurs in *Helix* (Krijgsman, 1929; Rosen, 1941; van Weel, 1961), although its role in feeding or digestion has been little studied. However, a pinocytic mechanism, permitting active absorption of dissolved food substances, would be a more attractive one than simple "diffusion," the process suggested by earlier investigators (Hirsch, 1915; Jordan and Bege-mann, 1921). There is no question that the first stage of digestion in the snail depends on a large number of enzymes acting in the gut lumen (Holden and Tracey, 1950; van Weel, 1961). Such extracellular enzymic activity could serve to break down ingested food initially, thereby permitting further intracellular digestion to take place.

Our present study does not test the possibility that migration of the clear colorless granules to the luminal border of the secretory-resorption cells during feeding could be related to discharge of enzymes into the lumen. The presence of increased E-600-resistant esterase activity at the luminal border during feeding might represent a stage in the release of proteolytic enzymes into the glandular lumen. It would seem, however, that initially extracellular digestion in the gut of an herbivorous species such as *Helix* would be brought about primarily by carbohydrases, not by enzymes more specifically suited to protein hydrolysis. It has been suggested (van Weel, 1961) that the salivary glands chiefly produce carbohydrases, and even that some of these enzymes are not produced by the animal itself (Florkin and Lozet, 1949; Jeuniaux, 1954). Once food has reached the gut and digestion begun, however, succeeding steps must involve some degree of intracellular digestion, especially of protein. Activity of the intracellular hydrolases described in the present study would appear to be well suited for intracellular breakdown of plant protein.

SUMMARY AND CONCLUSIONS

1. Cytochemical visualization methods for activity of acid phosphatase, β -glucuronidase, aminopeptidase and non-specific and E-600-resistant esterases were

applied to digestive gland tissue from starved and feeding *Helix pomatia*. Other cytochemical methods used included Baker's acid hematein for phospholipid and the periodic acid Schiff method.

2. Calcareous cells stained only for acid phosphatase activity, by both lead-salt and an azo dye method. Calcium granules within these cells did not stain with the azo dye method while false-positive reactions in the granules were always obtained with the lead-salt method. Some increase in enzymic activity was detected in the cytoplasm of feeding animals. Secretory-resorption (SR) cells showed little activity for acid phosphatase.

3. The yellow granules in SR cells stained for β -glucuronidase activity in both starved and feeding animals. After feeding, SR cells showed an increase in enzymic activity, both in granules and more diffusely in the cytoplasm. Although some aminopeptidase activity was present, insignificant differences in location and intensity of the enzyme were detected.

4. SR cells stained intensely for non-specific esterase activity. Cytoplasmic staining for this class of enzymes was intense in feeding animals. Treatment with the inhibitor E-600 resulted in loss of cytoplasmic staining; activity persisted in yellow granules in both starved and feeding animals. Some activity was detected in small, colorless granules.

5. Phospholipid was detected in peripheral regions of the SR cells from starved and feeding animals. The reaction was diffuse in starved animals but concentrated at the luminal border of the SR cells in fed animals. Yellow granules of SR cells were periodic acid Schiff-positive. Especially in fed animals, periodic acid Schiff granules appeared at the luminal border of SR cells. Starved animals fed on lettuce leaves impregnated with horse-radish peroxidase showed accumulation of enzyme in vacuoles closely associated with the yellow granules.

6. The observations extend the concepts, advanced by earlier workers, that granular components of the secretory-resorption cells play a significant role in digestion in the snail. The location of activity of the several hydrolases studied, and the alterations in response to feeding, suggest that these classes of enzymes and the granules form a functioning unit in the physiology of intracellular digestion in *Helix*.

LITERATURE CITED

- ARVY, L., 1962. Histochemie des enzymes impliqués dans la digestion, dans la série animale. *Handbk. Histochem.* VII, 2: 154-303.
- BAKER, J. R., 1946. Histochemical recognition of lipine. *Quart. J. Micr. Sci.*, 87: 441-470.
- BILLET, F., 1954. The β -glucuronidase of the Roman snail (*Helix pomatia*). *Biochem. J.*, 57: 159-162.
- BILLET, F., AND S. M. MCGEE-RUSSELL, 1955. The histochemical localization of β -glucuronidase in the digestive gland of the Roman snail (*Helix pomatia*). *Quart. J. Micr. Sci.*, 96: 35-48.
- BIRNS, M., 1960. The localization of acid phosphatase activity in the amoeba, *Chaos chaos*. *Exp. Cell Res.*, 20: 202-205.
- BURSTONE, M. S., 1958. Histochemical demonstration of acid phosphatase with naphthol-AS phosphates. *J. Nat. Cancer Inst.*, 21: 523-540.
- BURSTONE, M. S., AND J. E. FOLK, 1956. Histochemical identification of aminopeptidase. *J. Histochem. Cytochem.*, 4: 217-226.
- DODGSON, K. S., AND G. M. POWELL, 1959. Studies on sulphatases. 26. Arylsulphatase activity in the digestive juice and digestive gland of *Helix pomatia*. *Biochem. J.*, 73: 666-671.

- DEDUVE, C., 1959. Lysosomes, a new group of subcellular particles. In "Subcellular Particles" (ed. by T. Hayashi), pp. 128-158. Ronald Press, New York.
- FISHMAN, W. H., AND J. R. BAKER, II, 1956. Cellular localization of β -glucuronidase in rat tissue. *J. Histochem. Cytochem.*, **4**: 570-587.
- FLORKIN, M., AND F. LOZET, 1949. Origine bactérienne de la cellulase du contenu intestinal de l'escargot. *Arch. int. Physiol.*, **57**: 201-207.
- GOLDFARB, S., AND T. BARKA, 1960. Cytological localization of β -glucuronidase. *J. Histochem. Cytochem.*, **8**: 226-227.
- GOMORI, G., 1952. Microscopic Histochemistry. Chicago: University of Chicago Press.
- GUARDABASSI, A., AND E. FERRERI, 1953. Istofisiologia dell'apparato digerente di *Helix pomatia*. *Arch. Zool. Ital.*, **38**: 63-156.
- HESS, R., AND A. G. E. PEARSE, 1958. The histochemistry of indoxylesterase of rat kidney with special reference to its cathepsin-like activity. *Brit. J. Exp. Path.*, **39**: 292-299.
- HIRSCH, G. C., 1915. Ernährungsbiologie fleischfressender Gastropoden. *Zool. Jahrb. Physiol.*, **35**: 357-504.
- HOLDEN, M., AND M. V. TRACEY, 1950. A study of enzymes that can break down tobacco-leaf components. 2. Digestive juice of *Helix* on defined substrates. *Biochem. J.*, **47**: 407-414.
- HOLT, S. J., 1956. The value of fundamental studies of staining reactions on enzyme histochemistry with reference to indoxyl methods for esterases. *J. Histochem. Cytochem.*, **4**: 541-554.
- JARRIGE, P., AND R. HENRY, 1952. Étude de l'activité glucuronidasique du suc digestif d'*Helix pomatia* L. et de sa phénolsulfatase. *Bull. Soc. Chim. Biol.*, **34**: 872-885.
- JEUNIAUX, C., 1954. Sur la chitinase et la flore bactérienne intestinale des mollusques gastéropodes. *Bull. Acad. Roy Belg.*, **CI**, Sci. Mem. 28. F7.
- JORDAN, H. J., AND H. BEGEMANN, 1921. Ueber die Bedeutung des Darmes von *Helix pomatia*. *Zool. Jahrb. Physiol.*, **38**: 565-582.
- KRIJGSMAN, B. J., 1925. Arbeitsrhythmus der Verdauungsdrüsen bei *Helix pomatia*. I. *Zeitschr. vergl. Physiol.*, **2**: 264-296.
- KRIJGSMAN, B. J., 1929. Arbeitsrhythmus der Verdauungsdrüsen bei *Helix pomatia*. II. *Zeitschr. vergl. Physiol.*, **8**: 187-280.
- LEHRER, G. M., AND L. ORNSTEIN, 1959. A diazo coupling method for the electron microscopic localization of cholinesterase. *J. Biophys. Biochem. Cytol.*, **6**: 399-406.
- NAKAZIMA, M., 1956. On the structure and function of the midgut gland of mollusca, with a general consideration of the feeding habits and systematic relations. *Jap. J. Zool.*, **11**: 469-566.
- NOVIKOFF, A. B., 1959. Lysosomes and the physiology and pathology of cells. *Biol. Bull.*, **117**: 385.
- NOVIKOFF, A. B., AND E. ESSNER, 1960. The liver cell: some new approaches to its study. *Amer. J. Med.*, **29**: 102-131.
- ROSE, G. G., 1957a. Microkinetospheres in VP satellites of pinocytic cells observed in tissue culture of Gey's strain HeLa with phase contrast cinematographic techniques. *J. Biophys. Biochem. Cytol.*, **3**: 697-704.
- ROSE, G. G., 1957b. Observations on the dynamics of pinocytic and variant pinocytic (VP) cells in Gey's human malignant epidermoid strain HeLa. *Texas Repts. Biol. Med.*, **15**: 313-331.
- ROSEN, B., 1941. Intrazelluläre Verdauung bei *Helix pomatia* L. *Zool. Jahrb. Abt. f. allg. Zool. u. Physiol.*, **60**: 241-252.
- ROSENBAUM, R. M., 1962. The intracellular localization of enzymes. *Handbk. Histochem.*, **VII**, **4**: 1-108.
- ROSENBAUM, R. M., AND C. I. ROLON, 1960. Intracellular digestion and hydrolytic enzymes in the phagocytes of planarians. *Biol. Bull.*, **118**: 315-323.
- ROSENBAUM, R. M., AND C. I. ROLON, 1962. Species variability and the substrate specificity of intracellular acid phosphatases: a comparison of the lead-salt and azo-dye methods. *Histochem.*, **3**: 1-16.
- ROSENBAUM, R. M., AND M. WITTNER, 1962. Intracytoplasmic particles and enzymes associated with feeding and digestion in *Paramecium caudatum*. The possible role of the neutral red granules. *Arch. f. Protistenk.*, **106**: 223-240.

- SEAMAN, G. R., 1961. Acid phosphatase activity associated with phagotrophy in the ciliate *Tetrahymena*. *J. Biophys. Biochem. Cytol.*, 9: 243-245.
- STRAUS, W., 1959. Regional cytochemical identification of phagosomes in various tissues of the rat and their differentiation from mitochondria by the peroxidase method. *J. Biophys. Biochem. Cytol.*, 5: 192-204.
- STRAUS, W., 1961. Cytochemical observations on the transport of intravenously injected horse-radish peroxidase and the development of phagosomes in the cells of the kidney of the rat. *Exp. Cell Res.*, 22: 282-291.
- THIELE, G., 1953. Vergleichende Untersuchungen über den Feinbau und die Funktion der Mitteldarmdrüse einheimischer Gastropoden. *Zeitschr. Zellforsch.*, 38: 87-138.
- VAN WEEL, P. B., 1961. The comparative physiology of digestion in molluscs. *Amer. Zool.*, 1: 245-252.
- WEISS, L. P., AND D. W. FAWCETT, 1953. Cytochemical observations on chicken monocytes, macrophages and giant cells in tissue culture. *J. Histochem. Cytochem.*, 1: 47-65.